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The Spinocerebellar Ataxia 8 Noncoding RNA Causes Neurodegeneration and Associates with Staufen in *Drosophila*

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Summary

Spinocerebellar Ataxia 8 (SCA8) appears unique among triplet repeat expansion-induced neurodegenerative diseases because the predicted gene product is a noncoding RNA. Little is currently known about the normal function of SCA8 in neuronal survival or how repeat expansion contributes to neurodegeneration. To investigate the molecular context in which SCA8 operates, we have expressed the human SCA8 noncoding RNA in *Drosophila*. SCA8 induces late-onset, progressive neurodegeneration in the *Drosophila* retina. Using this neurodegenerative phenotype as a sensitized background for a genetic modifier screen, we have identified mutations in four genes: *staufen*, *muscleblind*, *split ends*, and *CG3249*. All four encode neuronally expressed RNA binding proteins conserved in *Drosophila* and humans. Although expression of both wild-type and repeat-expanded SCA8 induce neurodegeneration, the strength of interaction with certain modifiers differs between the two SCA8 backgrounds, suggesting that CUG expansions alter associations with specific RNA binding proteins. Our demonstration that SCA8 can recruit Staufen and that the interaction domain maps to the portion of the SCA8 RNA that undergoes repeat expansion in the human disease suggests a specific mechanism for SCA8 function and disease. Genetic modifiers identified in our SCA8-based screens may provide candidates for designing therapeutic interventions to treat this disease.

Results and Discussion

The most extensively studied triplet repeat expansion-induced neurodegenerative disorders include diseases such as Huntington's, in which extended polyglutamine tracts within the encoded protein appear responsible for pathogenesis [1]. A new class of degenerative disorders has recently emerged in which microsatellite repeat expansions within noncoding regions of the gene result in an altered RNA product that somehow exerts a dominant, deleterious effect [2]. Included in this category are Myotonic Dystrophy types 1 and 2 (DM1 [3] and DM2 [4]) and three Spinocerebellar Ataxias (SCAs) [5]: SCA8 [6–8], SCA10 [9], and SCA12 [10].

SCA8 stands apart from other members of this class because the CUG triplet repeat expansions occur within a transcript that is entirely noncoding [2, 6]. However, the mechanism of SCA8-mediated pathogenesis remains an area of intense debate [11, 12]. One hypothesis suggests that repeat expansion leads to a toxic RNA with impaired or altered cellular function [2]. Thus analogous to what has been proposed for DM1, alterations in secondary structure resulting from CUG repeat expansion might lead to abnormal interactions with specific RNA binding proteins, thereby titrating them away from other essential binding partners and leading to disease. Alternatively, because the SCA8 transcript is an endogenous antisense RNA that partially overlaps the *Kelch-like 1*(*KLHL1*) gene [13], repeat expansion might impair regulation of the *KLHL1* locus [7].

To investigate the molecular mechanisms underlying SCA8 function and pathogenesis, we expressed the human SCA8 noncoding RNA in the *Drosophila* eye. Exons D, C3, C2, and A from the human SCA8 cDNA [7] were placed under control of the UAS element [14] and multiple transgenic lines were generated for both the wild-type (SCA8[CTG9]) and CTG expanded (SCA8[CTG112]) forms. Although CTG repeat lengths of 110–250 have been associated with disease, not all individuals carrying an expanded *SCA8* allele develop SCA8 ataxia [8, 11]. Therefore, we used a CTG repeat configuration that is known to be pathogenic, $CTA_3CTG_5CCG_3CTG_{112}$, by generating the expanded *SCA8* allele directly from the genomic DNA of a patient whose inherited ataxia has been genetically linked to the SCA8 locus [6]. Similarly, the wild-type SCA8 cDNA was generated from the unexpanded allele of an unaffected member of the same family and has the repeat configuration CTA_9CTG_9 [6]. Genomic stability of the CTG repeats in the transgenic lines was confirmed by Southern hybridization (data not shown).

Using the *gmr*-GAL4 driver to induce expression in all photoreceptor neurons, we found that expression of SCA8(CTG9) and SCA8(CTG112) leads to disorganization of the ommatidia and mechanosensory bristles of the adult eye, with the central portion of the eye most severely affected (Figures 1A–1C). Multiple independent transgenic lines were examined and a range of rough-eye phenotypes was observed (Figure S1A). Furthermore, the rough-eye phenotype exhibited variable penetrance within a given line. For example, in the SCA8(CTG9) and SCA8(CTG112) lines described in this study, the majority of flies (80.2% and 73.4%, respectively) exhibit a moderate rough eye, while the rest have a milder phenotype (Figure S1C). RT-PCR analysis indicated comparable SCA8 expression levels in flies showing mild versus moderate phenotypes, suggesting the variability within a given line is not caused by instability of either the transgenes or the encoded RNAs (Figure S1D).

To investigate the cellular defects responsible for the abnormal external morphology, the eyes were sec-

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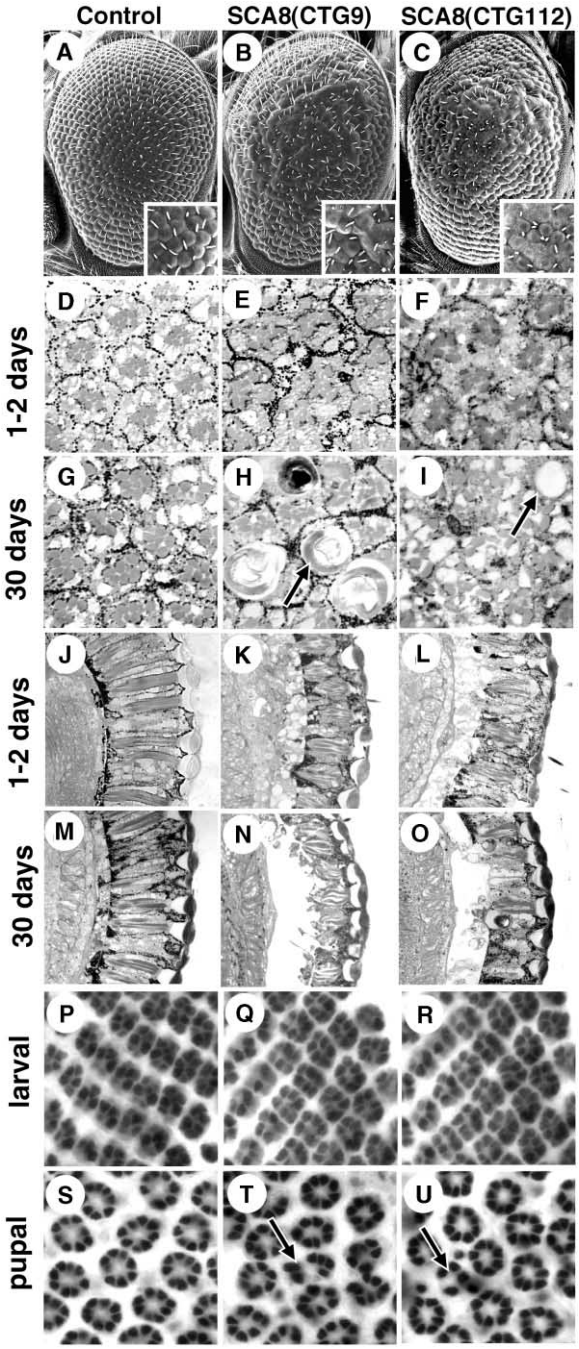

Figure 1. SCA8 Expression Leads to Age-Dependent, Progressive Neural Degeneration

(A–C) SEM of adult eyes. Insets show higher magnification of ommatidial field. (A) *gmr-GAL4/+* control. (B) *gmr-GAL4/+*; *UAS:SCA8 (CTG9)/+* and (C) *gmr-GAL4/+*; *UAS:SCA8(CTG112)/+* have comparable rough-eye phenotypes.

(D–I) Tangential sections of adult eyes. (D–F) 1- to 2-day-old flies. (G–I) 30-day-old flies. (D) *gmr-GAL4/+* control has a well-organized retina. (E) *gmr-GAL4/+*; *UAS:SCA8(CTG9)/+* and (F) *gmr-GAL4/+*; *UAS:SCA8(CTG112)/+* retinas exhibit mild disorganization with loss of photoreceptor neurons and pigment cells. (G) *gmr-GAL4/+* control shows only mild retinal deterioration after 30 days. (H) *gmr-GAL4/+*; *UAS:SCA8(CTG9)/+* and (I) *gmr-GAL4/+*; *UAS:SCA8 (CTG112)/+* flies show progressive retinal degeneration with photoreceptor neurons becoming rod shaped and fragmented, leading to formation of vacuoles (arrows).

tioned. In the control (Figure 1D), this analysis reveals the uniform cellular organization of the ommatidia, each of which contains eight photoreceptor neurons surrounded by cone cells and pigment cells. Ectopic expression of wild-type and expanded SCA8 leads to loss and disorganization of the photoreceptor neurons and pigments cells (Figures 1E and 1F). We next asked whether SCA8-associated neurodegeneration is age dependent and progressive by comparing tangential retinal sections from young (1–2 days) and old (30 days) adult flies expressing SCA8 to age-matched controls. While the retinas of control flies expressing the *gmr*-GAL4 driver alone exhibit a mild age-dependent deterioration (Figures 1D and 1G), the degeneration is much more pronounced when SCA8 transgenes are expressed (Figures 1E, 1H, 1F, and 1I). Frontal sections of the brain and retina further confirm the progressive nature of SCA8-induced neurodegeneration in *Drosophila* adults (Figures 1J–1O).

Staining of the larval eye discs with the neuronal marker ELAV revealed no apparent disruption in recruitment and differentiation of the photoreceptor neurons (Figures 1P–1R). Degeneration of the mature photoreceptors appears to initiate during pupal stages, as evidenced by the mild neuronal loss seen in SCA8 expressing pupal eye discs (Figures 1S–1U). Thus, the rough-eye phenotype observed in adults reflects a later degeneration of mature neurons as opposed to an early developmental defect.

We next asked whether despite the comparable overexpression phenotypes, there might be differences in expression levels and/or localization of the wild-type versus expanded SCA8 transcripts. Although not a quantitative assay, in situ hybridization revealed comparable expression from SCA8(CTG9) and SCA8(CTG112) transgenes in larval eye discs (Figure S2A). Similarly, semiquantitative RT-PCR analysis from adult heads revealed roughly comparable expression levels, although we consistently observed a 1.3-fold increase in transcripts produced from SCA8(CTG9) versus SCA8(CTG112) transgenes (Figure S1D). Because knowledge of transcript localization has important implications for understanding the function of SCA8 and has not been previously reported in human tissues, we expressed the SCA8 transgenes in the larval salivary gland and associated fat body. We found that both the SCA8(CUG9) and the SCA8(CUG112) transcripts localized predominantly

(J–O) Frontal sections of adult retinas. (J–L) 1- to 2-day-old flies. (M–O) 30-day-old flies. (J) The retinas of *gmr-GAL4/+* control flies are normal. Mild thinning of the retina is seen in (K) *gmr-GAL4/+*; *UAS:SCA8(CTG9)/+* and (L) *gmr-GAL4/+*; *UAS:SCA8(CTG112)/+* flies. (M) No retinal deterioration is observed in *gmr-GAL4/+* controls. (N) *gmr-GAL4/+*; *UAS:SCA8(CTG9)/+* and (O) *gmr-GAL4/+*; *UAS:SCA8(CTG112)/+* exhibit retinal thinning and degeneration with increased age.

(P–U) Anti-*elav* staining reveals the recruitment of photoreceptor neurons in larval (P–R) and pupal (S-U) eye imaginal discs. (P) *gmr-GAL4/+* control. Photoreceptor recruitment in (Q) *gmr-GAL4/+*; *UAS:SCA8(CTG9)/+* and (R) *gmr-GAL4/+*; *UAS:SCA8(CTG112)/+* is normal. (S) *gmr-GAL4/+* control. (T) Loss of photoreceptors (arrows) seen in midpupal eye discs from (T) *gmr-GAL4/+*; *UAS:SCA8 (CTG9)/+* and (U) *gmr-GAL4/+*; *UAS:SCA8(CTG112)/+* flies.

Table 1. Modifiers of SCA8 Identified in P Element Insertion Screen

P Insertion Line	Modifier	Gene	Function
l(2)k02512	Enhancer	*lolal*	RNA Polymerase II transcription factor
l(2)k04203	Enhancer	*Aats-thr*	Threonine-tRNA ligase, alpha-L RNA-binding motif
l(2)k07104	Enhancer	*hoip*	mRNA splicing, RNA binding
l(2)k07207	Enhancer	*Vha36*	Hydrogen-exporting ATPase, proton transport
l(2)k07433	Enhancer	*CG33130*	Calpolin homology domain, signal trannsduction
l(2)k08015	Enhancer	*CG10228*	mRNA cleavage factor complex
l(2)k08316	Enhancer	*px*	PHD Zinc finger motif
l(2)k10217	Enhancer	*CG15625/CG3036*	P-element inserted between *CG15625/CG3036*
l(2)k10809	Enhancer	*Ate*	arginyltransferase
l(2)k11904	Enhancer	*ttv*	Acetylglucosaminyltransferase
l(2)k03902	Suppressor	*Cas*	Importin-alpha export receptor
l(2)k04308	Suppressor	*gem*	Transcription factor with SAM/pointed domain
l(2)k06709	Suppressor	*RnrL*	Ribonucleoside-diphosphate reductase
l(2)k07619	Suppressor	*hrg*	Polynucleotide adenyltransferase, RNA binding
l(2)k07824	Suppressor	*CG7989*	Contains Trp-Asp(WD) repeat
l(2)k07834	Suppressor	*Psc*	DNA binding, chromatin silencing
l(2)k09854	Suppressor	*GstS1*	Glutathione S transferase
l(3)07041	Enhancer	*Eip75B*	Ligand-dependent nuclear receptor
l(3)j1E7	Enhancer	*l(3)j1E7*	Unknown
BG02228	Enhancer	*Sac1*	Polyphosphoinositide phosphatase
l(3)L1820	Suppressor	*Parp-E*	NAD ADP-ribosyltransferase
l(3)L3101	Suppressor	*mod(mdg4)*	BTB/POZ domain, RNA Polymerase II transcription factor
fs(3)07084	Suppressor	*S6K*	Ribosomal protein S6 Kinase
l(3)09070	Suppressor	Unknown*	
l(3)10547	Suppressor	*Int6*	Translational initiation factor
l(3)rK509	Suppressor	*CG2503*	Signal transduction

The assignment of the genes and probable functions are based on Flybase annotation. For insertions not annotated, the inverse PCR sequence from Flybase was blasted to map the gene closest to the site of insertion.
*Could not map insertion as the inverse PCR sequence was too short.

to the fat body nuclei (Figure S2B). Thus, CTG repeat expansion does not dramatically alter the levels or nuclear localization of SCA8 transcripts, at least in the context of our *Drosophila* system.

How might overexpression of SCA8 lead to neurodegeneration in the fly eye? We hypothesize that high levels of SCA8 transcripts may titrate away critical RNA binding proteins or other factors required for neuronal survival, resulting in retinal degeneration. Because the phenotypes associated with overexpression of SCA8(CTG9) and SCA8(CTG112) are similar, neuronal degeneration may simply reflect the elevated SCA8 transcript accumulation, regardless of CUG repeat length. Alternatively, repeat expansion could alter SCA8's ability to interact productively with proteins that normally complex with the wild-type transcript or could result in inappropriate associations with new proteins, in both cases triggering neuronal degeneration.

We have used the rough-eye phenotype that results from overexpression of SCA8(CTG112) as a sensitized genetic background in which to test these models. We first screened a collection of 957 lethal P element insertion lines for those capable of dominantly enhancing or suppressing SCA8-induced neurodegeneration. Isolation of mutations in several RNA binding proteins as dominant modifiers of SCA8 (Table 1) encouraged us to perform a more comprehensive survey of RNA binding proteins. We therefore used a candidate gene approach to screen a collection of mutations in 22 known RNA binding proteins (Table 2). Mutations that interacted with the *gmr*-GAL4 driver line alone were deemed nonspecific (data not shown). This targeted screen recovered mutations in four genes; three enhancers, *muscleblind* [15, 16], *split ends* [17–19], and *staufen* [20–22]; and one suppressor, *CG3249*, which encodes a putative PKA anchor protein (PKAAP) [23, 24] with a KH-type RNA binding motif (Figures 2A–2I and Figure 3). None of the modifiers exhibit dominant eye phenotypes on their own, indicating that interaction with SCA8 is synergistic. It is possible that our screen failed to identify critical SCA8-interacting genes simply because they are not expressed in the *Drosophila* photoreceptor neurons. However, the fact that not all neuronally expressed genes tested were identified as modifiers (for example, *elav*, *musashi*, and *fragile-X*) indicates a degree of specificity and selectivity to the interactions (Table 2).

For *muscleblind*, *split ends*, and *staufen*, enhancement was observed with multiple loss-of-function alleles (Table 2 and data not shown). In the case of *CG3249*, the allele used was a P element engineered to activate expression of the gene immediately downstream of the insertion site. Thus, suppression of SCA8-mediated neurodegeneration resulted from coexpression of PKAAP. Further confirming the specificity of this interaction, a loss-of-function allele of *CG3249* enhanced the SCA8 rough-eye phenotype (Figures 2G and 2H). We also asked whether the genetic interactions might reflect an alteration in SCA8 RNA stability. However, RT-PCR analysis did not reveal any obvious changes in SCA8 RNA levels (Figure 2J).

Next, we asked whether modifiers isolated in our SCA8(CTG112) based screen interacted with the SCA8(CTG9) sensitized background (Figure 3). Two of the four modifiers exhibited different interaction strengths with

Table 2. RNA Binding Protein Mutants Screened for Interactions with SCA8

Gene	Function	Loss-of-Function Alleles	Overexpression	Modification
apontic	RNA polymerase II transcription factor	apt^{03041}, $aptk^{15608}$	apt^{EP2339}	No
aret	negatively regulates oskar mRNA	$aret^{01284}$, $aret^{BG006}$		No
B52	pre-mRNA splicing factor	$B52^{s2249}$		No
couch potato	RNA-binding	cpo^{01432}		No
egalitarian	helicase	egl^{1286}	egl^{EP0938}	No
Eukaryotic initiation factor 4E	translation initiation factor	$eIF\text{-}4E^{07238}$	$eIF\text{-}4E^{EP0568}$	No
elav	mRNA-binding	$elav^{G0031}$, $elav^{C15}$, $elav^{4}$		No
Eukaryotic initiation factor 4A	RNA helicase	$eIF\text{-}4a^{k01501}$	$eIF\text{-}4a^{EP1011}$	No
heph2	pre-mRNA splicing factor	$heph^{2}$		No
muscleblind	Nucleic acid binding, binds to CUGn	mbl^{k01212}, mbl^{k07103}, mbl^{E27}, mbl^{E16}		Enhancer
musashi	negative regulator of translation	msi^{1}, msi^{2}		No
mushroom-body expressed	single stranded RNA-binding	mub^{01038}		No
no on off transient A	mRNA splicing factor	$nonA^{4b18}$		No
oo18 RNA-binding protein	mRNA polyadenylation	orb^{dec}		No
CG3249 (PKAAP)	Protein kinase A anchor protein		$CG3249^{EP1400}$	Suppressor
		$CG3249^{KG02745}$		Enhancer
pumilio	3′UTR binding	pum^{3}, pum^{01688}		No
qkr-58-E	KH domain RNA binding protein		EP2103	No
CG9381/RNA-binding protein S1	RRM domain splicing factor		EP1082	No
split ends	RNA binding protein	$spen^{XFM911}$, $spen^{AH393}$		Enhancer
staufen	Double stranded RNA binding	$stau^{1}$, $stau^{D3}$, $stau^{ry9}$		Enhancer
sex-lethal	mRNA splicing factor	sxl^{fs3}		No
fragile-X	Translational regulator	Df(3R)by62, $dfmr^{del50}$		No

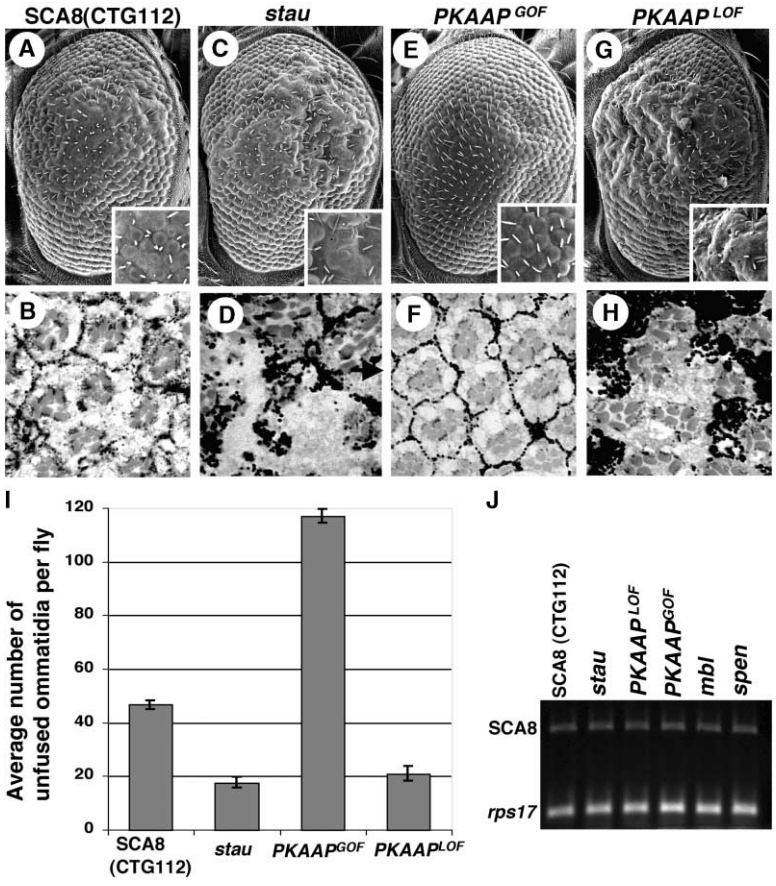

Figure 2. Mutations in RNA Binding Proteins Modify *SCA8(CTG112)*-Induced Neurodegeneration.

(A, C, E, and G) SEM of adult eyes. Insets show higher magnification of ommatidial field. (B, D, F, and H) Tangential sections of adult eyes. (A and B) *gmr-GAL4/+*; *UAS:SCA8(CTG112)/+*. (C and D) *gmr-GAL4/* $stau^{D3}$*; UAS:SCA8(CTG112)/+* flies have an enhanced rough-eye phenotype with loss of photoreceptor neurons. (E and F) Overexpression of *CG3249*, $CG3249^{EP1440}$*/+;gmr-GAL4/+*; *UAS:SCA8(CTG112)/+*, suppresses the *SCA8(CTG112)* rough-eye phenotype and restores ommatidial integrity. (G and H) Conversely, loss of *CG3249*, $CG3249^{KG02745}$*/+;gmr-GAL4/+*; *UAS:SCA8(CTG112)/+*, enhances the SCA8 degenerative phenotype.

(I) Severity of the SCA8(CTG112) modifier phenotypes was quantified by counting the average number of unfused ommatidia within a central field of ~200 ommatidia per fly. For each genotype, ommatidia from three flies were examined by SEM. The alleles used for the different modifiers are $stau^{D3}$, $CG3249^{EP1440}$ (*PKAAP*^{GOF}), and $CG3249^{KG02745}$ (*PKAAP*^{LOF}).

(J) None of the modifiers influenced SCA8 RNA stability as shown by semiquantitative RT-PCR analysis from adult heads.

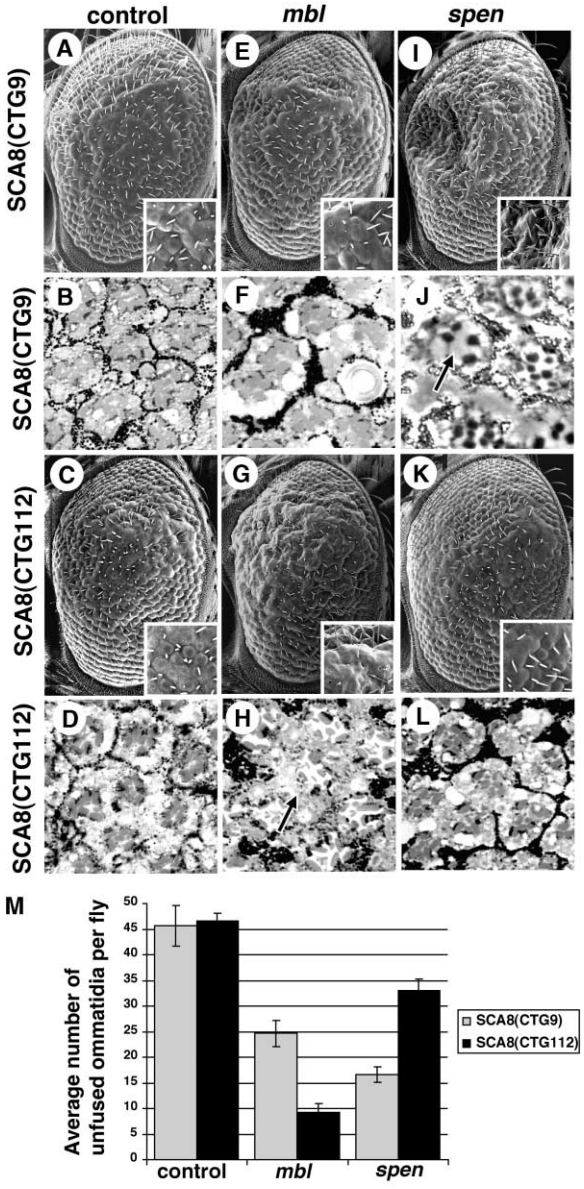

Figure 3. Genetic Modifiers Exhibit Different Interaction Strengths with *SCA8(CTG112)* versus *SCA8(CTG9)*

(A, C, E, G, I, and K) SEM of adult eyes. Insets show higher magnification of ommatidial field.

(B, D, F, H, J, and L) Tangential sections of adult eyes.

(A and B) *gmr-GAL4/+*; *UAS:SCA8(CTG9)/+*. (C and D) *gmr-GAL4/+*; *UAS:SCA8(CTG112)/+*. (E and F) Mild enhancement of the SCA8-induced phenotype by *muscleblind* in *gmr-GAL4/mbl^{E16}*;UAS:SCA8(CTG9)/+ adult eye. (G and H) Increased enhancement of *gmr-GAL4/mbl^{E16}*; *UAS:SCA8(CTG112)/+* is noted by the increased loss of photoreceptors and disorganization of pigment cells (arrow) as compared to *SCA8(CTG9)* where clusters of photoreceptors can be seen (refer to F). (I and J) The enhancement of the SCA8-induced phenotype and loss of photoreceptors (arrow) is more pronounced in *gmr-GAL4/spen^{AH393}*; *UAS:SCA8(CTG9/+)* as compared to (K and L) *gmr-GAL4//spen^{AH393}*; *UAS:SCA8(CTG112)/+*) adult eyes.

(M) The different strengths of genetic interaction of SCA8(CTG9) and SCA8(CTG112) with *muscleblind* and *spen* loss-of-function alleles (*mbl^{E16}* and *spen^{AH393}*) were quantified. *mbl* interacts more strongly with SCA8(CTG112) than with SCA8(CTG9), whereas *spen* interacts more strongly with SCA8(CTG9) than with SCA8(CTG112). The average number of unfused ommatidia within a central field of ~200 ommatidia per fly was counted. For each genotype, ommatidia from three flies were examined by SEM.

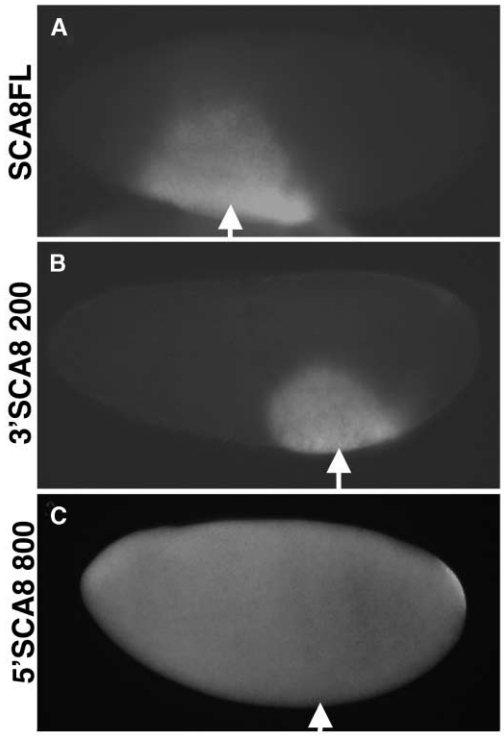

Figure 4. Injected SCA8 RNA Recruits Staufen In Vivo

(A–C) Embryos injected with unlabeled SCA8 RNA were stained with anti-Staufen. Injection site is marked with an arrow. (A) Full-length SCA8 sense RNA (~1 kb in length, referred to as SCA8-FL) recruits Staufen to the site of injection (arrow). (B) A similar result is seen with the most terminal ~200 bases of the SCA8 RNA (arrow). (C) Injection of an ~800 base fragment of SCA8 lacking the 3′ CUG-containing region no longer recruits Staufen.

SCA8(CTG112) versus SCA8(CTG9). Mutations in *muscleblind* enhanced the SCA8(CTG112) phenotype more strongly than observed with SCA8(CTG9) (Figure 3M and compare Figures 3E to 3G and 3F to 3H). Second, in the case of *spen*, a weaker interaction was observed with SCA8(CTG112) than with SCA8(CTG9) (Figure 3M and compare Figures 3I to 3K and 3J to 3L). These results indicate that although the neurodegeneration that results from expression of either SCA8(CTG112) or SCA8(CTG9) is histologically similar (Figures 3A–3D), there may be differences in the underlying molecular causes of the phenotypes.

To begin to validate the genetic interactions uncovered in our screen, we have extended our analyses of *staufen*, which encodes a conserved RNA binding protein that mediates mRNA localization and transport both in the oocyte and in the nervous system [20–22, 25, 26]. We exploited an in vivo RNA binding assay developed by Ferrandon et al., [21], which involved injecting SCA8 RNA into early embryos and looking for recruitment of Staufen. Unlabeled sense-strand SCA8(CUG9) RNA recruited Staufen to the site of injection (Figure 4A). We

were unable to test SCA8(CUG112) because the increased CUG repeat length inhibited in vitro RNA synthesis.

In order to map the region of SCA8 required for interacting with Staufen, we injected two smaller fragments, a 3′ ~200 nucleotide RNA, which includes the CUG repeat region, and a 5′ ~800 nucleotide RNA (Experimental Procedures). Injection of the smaller CUG-containing RNA recruited Staufen efficiently (Figure 4B), whereas no obvious recruitment was seen with the 5′ ~800 nucleotide RNA (Figure 4C). Identical results were observed when FITC-labeled SCA8 RNA was injected (Figure S3). Thus, the CUG containing 3′ region of the SCA8 noncoding RNA appears sufficient to mediate interactions with Staufen.

The results of our targeted genetic modifier screen provide the first description of the molecular context within which SCA8 may function. Identification of *muscleblind* alleles as enhancing SCA8(CTG112) more strongly than SCA8(CTG9) served as proof of principle because Muscleblind has been shown previously to bind CUG repeats in the 3′UTR of DM1, with the strength of interaction increasing with increased CUG repeat length [27]. *muscleblind* encodes a conserved nuclear zinc-finger domain protein and, in *Drosophila*, is required for muscle development and photoreceptor differentiation [15, 16]. Further supporting the premise that increased CUG repeats alters SCA8 function and interaction with its normal binding partners, *spen* mutations enhanced SCA8(CTG112) less strongly than SCA8(CTG9). *spen* encodes the founding member of a family of RNA recognition motif (RRM) containing nuclear proteins. *spen* mutant phenotypes include defects in the central nervous system and photoreceptors, consistent with its enriched expression in neural tissues [17–19, 28]. Although Spen family members have been implicated in multiple processes in both flies and mammals [29], little is known about possible RNA targets. Our results provide a potential link between a critical signaling molecule, Spen [28], and the essential but poorly understood processes that maintain neuronal integrity.

The putative Protein kinase A anchor protein [23, 24], identified as a suppressor when overexpressed and an enhancer when its dosage is reduced, belongs to a group of structurally diverse proteins that bind the regulatory subunit of PKA, confining the enzyme to discrete locations within the cell. Perhaps the PKAAP identified in our screen, which also contains a KH-motif for RNA binding, similarly sequesters SCA8, thereby preventing deleterious interactions with other RNA binding proteins and leading to suppression of the neurodegenerative phenotype. $CG3249^{EP1400}$, the allele identified in our screen, has been previously recovered from a screen for genes controlling motor axon guidance and synaptogenesis in *Drosophila*, consistent with a role in differentiated neurons [30].

Our demonstration that Staufen, identified as an enhancer in our screens, is recruited by SCA8, suggests that SCA8-Staufen associations, whether direct or indirect, are likely to be important for normal neuronal function and survival. The finding that the interaction is mediated by the CUG repeat containing the ~200 nucleotide terminal portion of SCA8 RNA leads us to speculate that either the ability to interact with Staufen or the functional consequences of such an interaction could be compromised by CUG repeat expansion, leading to disease. Staufen has been shown to interact with the actin cytoskeleton and to be required for proper localization and targeting of RNAs [25, 31]. For example, rat *staufen* appears necessary for transport of RNAs to neuronal dendrites [26, 32]. In *Drosophila*, Staufen binds to the 3′UTR of *prospero* and mediates its proper localization in the larval neuroblasts [22, 25, 31]. More recently, *staufen* has been identified as a necessary component of long-term memory formation in *Drosophila* [33].

In conclusion, it is important to emphasize that the connection between repeat expansion and SCA8 ataxia remains a matter of contentious debate. Determining the clinical relevance of CTG repeat expansions to those patients who carry them will probably not be possible until the molecular context underlying SCA8 function in both normal and pathogenic contexts is more fully understood [12]. The set of evolutionarily conserved and neuronally expressed RNA binding proteins identified in our experiments provides a critical molecular handle that once validated in mammalian model systems, should facilitate resolution of the controversy regarding the role of CTG repeat expansion in SCA8-associated neurodegeneration.

Supplemental Data

Supplemental Data including Experimental Procedures and three figures (S1–S3) describing the variability in SCA8 expression levels, SCA8 subcellular localization, and the Staufen colocalization assay using fluorescently labeled SCA8 RNA are available at http://www.current-biology.com/cgi/content/full/14/4/302/DC1/.

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